Design and Optimization of a Temperature-Stable Dry Powder BCG Vaccine

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Purpose
The current vaccine against Mycobacterium tuberculosis, Bacille Calmette-Guérin (BCG), has a highly variable efficacy rate of 0-80% in terms of protecting against infection. BCG is a live, attenuated form of Mycobacterium bovis given by the intradermal route. Losses in viability from exposure to extreme temperatures (high and below freezing) during storage and transportation remain a significant obstacle to BCG and other live vaccines. Additionally, our laboratory and others have shown that administering BCG by the pulmonary route greatly increases its efficacy. Here we show the development and optimization of a thermostable BCG dry powder vaccine. Furthermore, we characterize the live vaccine powder for its shelf-life at different temperature and humidity conditions and its suitability for pulmonary administration.

Methods
A factorial design of experiment was performed using Design-Expert® software to optimize the following excipient ratios: L-leucine, bovine serum albumin (BSA), polyvinylpyrrolidone (PVP), mannitol, and trehalose. These excipients were spray dried with a Buchi mini-spray dryer B-290 and the following spray parameters were used: Inlet temperature 150±5 °C, outlet temperature 50±5 °C, aspirator rate 100%, pump flow rate 10% (corresponding to 4 mL/min), and air flow rate 742 L/hr. Powders were characterized for size, yield, water retention, water uptake, and glass transition temperature (Tg). Three optimal dry powder carriers were selected based on stability and powder flow characteristics, and were spray-dried with live BCG. BCG dry powders were stored for 6 months at the following conditions: -20 °C, 4 °C, 25 °C (relative humidity (RH) of 40% and 60%), and 40 °C (RH 75%), and were assessed for bacterial viability. Powders were compared to a previously published formulation of BCG dry powder which utilized only L-leucine for stability.

Results
The factorial design generated a total of 25 runs which were spray-dried, characterized, and recorded. Figure 1 shows the effect of excipient concentration on size, yield, water retention, water uptake, and Tg. Design Expert software generated three optimized excipient mixtures to spray dry with BCG based on the above parameters. Spray drying BCG resulted in an initial half log loss in bacterial viability. These formulations had an average geometric size of Dx(50)= 2.02±2.14 μm, with over 80% of particles being in the respirable range. Furthermore, these powders had lower residual moisture, were less hygroscopic than the leucine only BCG powder. Lastly, these formulations were stable at all environmental conditions tested for up to 6 months, whereas leucine-BCG powder showed higher loss. BCG dry powders will be further assessed for antigenicity and lung deposition in mice.

Conclusion
The temperature stability of live vaccines is critical to maintain its potency. Currently refrigeration is required for most vaccines. In spite of the cold chain, many vaccines are adversely affected during transport and storage every year due to exposure to high and freezing temperatures. Our data suggests that we have improved the overall characteristics of BCG dry powders, compared to the previously published BCG-leucine dry powder, by adding excipients which improve powder size for pulmonary delivery, limit moisture uptake, increase the amorphous nature, and improve the thermostability of BCG.